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Bouquet et al.

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(54) **IMMORTAL AVIAN CELLS**

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(56) **References Cited**

FOREIGN PATENT DOCUMENTS

0 242 272 A1 10/1987 (EP) .
WO 91/18971 12/1991 (WO) .
WO 92/10563 6/1992 (WO) .
WO 93/20200 10/1993 (WO) .

OTHER PUBLICATIONS

"The 12S adenoviral E1A protein immortalizes avian cells and interacts with the avina RB product", Guilhot et al, *Oncogene*, 1993, vol. 8, pp. 619–624.

"Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus", Naniche et al, *Journal of Virology*, Oct. 1993, pp. 6025–6032.

"Immortalization of human fibroblasts transformed by origin–defective simian virus 40", Neufeld et al, *Molecular and Cellular Biology*, Aug. 1987, pp. 2794–2802.

"Overexpression of human cyclin A advances entry into S phase", Rosenberg et al, *Oncogene* 1995, vol. 10, pp 1501–1509.

"Activation of Epstein–Barr virus latent genes protects human B cells from death by apoptosis", Gregory et al, *Nature*, Feb. 2, 1991, vol. 349, pp. 612–614.

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(57) **ABSTRACT**

The invention features apoptosis-resistant, non-transformant immortalised avian cells, in particular, avian tissues, i.e., other than blood or haematopoietic cells, particularly fibroblasts and epithelial cells, for instance embryos.

15 Claims, 1 Drawing Sheet

the microscope. The infectious titre is calculated by the KARBER method and is expressed by the logarithm of the inverse of the viral dilution which gives 50% CPE [titre= d+r/Nx(n+N/2)], where d is the dilution expressed in logs when all the wells are positive, r is the dilution ratio, N is the number of wells per dilution and n is the number of positive wells between 0 and 100%.

Results: The viral titres obtained are equivalent to those obtained on primary duck embryo cells.

EXAMPLE 5

Integration of the bcl-2 gene

A vector which permits expression of the bcl-2 gene under the control of the CMV (human cytomegalovirus) promoter is transfected into the TDF-2A and TCF-4.10 cells using conventional transfection methods (DMSO method described by Kawai and Nishizawa (1984), Mol. Cell. Biol. 4: 1172–1174 or lipofectamine method in accordance with the supplier's (GIBCO-BRL) recommendations).

After the transfected cells have been selected, expression of the Bcl-2 protein is detected by Western blotting.

The cells which express the Bcl-2 protein are then tested for their ability to survive under culture conditions in which an apoptosis process is observed (maintenance of the cells at confluence).

Thus, in the case of the TDF-2A bcl-2 cells, the apoptosis process engendered by the cells arriving at confluence is deferred by from 3 to 4 days as compared with the TDF-2A cells. An increase in cell density at confluence is observed in the TCF-4.10 bcl-2 cells as compared with the TCF-4.10 cells.

What is claimed is:

1. An avian cell line, comprising avian cells which are immortalized, but untransformed, the cells comprising, integrated into their genome, an antiapoptotic bcl-2 gene.

2. The avian cell line according to claim **1**, wherein it is obtained from cells of avian tissues.

3. The avian cell line according to claim **2**, wherein it is obtained from fibroblasts or epithelial cells.

4. The avian cell line according to claim **1**, wherein the cells comprise, integrated into their genome, the SV40 T+t gene.

5. The avian cell line according to claim **4**, wherein the SV40 T+t gene is under the control of the MTI promoter.

6. An immortal, untransformed avian cell line, which is selected from the group consisting of:

cell line TDF-2A bcl-2, which is deposited in the CNCM (Pasteur Institute National Collection of Microorganism Cultures) under reference number I-1709; cell line TCF-4.10, which is deposited in the CNCM under the reference number I-1710; and cell line TCF-4.10 bcl2, which is deposited in the CNCM under reference number I-1711.

7. Immortal avian cells which are obtained from the cell line according to claim **6**.

8. The cells according to claim **7**, wherein the cells express a heterologous nucleotide sequence.

9. The cells according to claim **8**, wherein the nucleotide sequence encodes a viral peptide, protein or glycoprotein or encodes protein molecules.

10. The cells according to claim **7**, wherein they are infected with a virus which is multiply in these cells.

11. The cells according to claim **7**, wherein they contain an anti-apoptotic gene selected from the group consisting of p19E1B from human adenovirus, LMP-1 from Epstein Barr virus, BHRF1 from Epstein Barr virus, ICP34.5 from herpes simplex virus and p35 from baculovirus.

12. The cells according to claim **7**, wherein the cells comprise a vector comprising a gene encoding viral receptor.

13. The cells according to claim **7**, wherein the cells comprise a vector comprising a gene encoding a cellular receptor for a virus.

14. The cells according to claim **7**, wherein the cells comprise a vector comprising a gene encoding an oncogene.

15. A method for producing viruses or a viral peptide, protein, glycoprotein, or protein molecules which comprises selecting a cell according to any one of claims **7** to **14**, inserting a nucleotide sequence into said cell, culturing said cell and expressing said nucleotide sequence so as to produce a virus or a viral peptide, protein, glycoprotein, or protein molecule.

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